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13. ABSTRACT (Maximum 200 Words) The Breast Cancer Training Program for Summer Undergraduates (BCTP-SU) has been established within the Eppley Cancer Research Institute of the University of Nebraska Medical Center (UNMC). The purpose of the BCTP-SU is to expand the Eppley's summer research program to add five additional undergraduates to train specifically in breast cancer research. Trainees participated in didactic and academic activities, including: 1) a 10 week, lab-intensive research project, mentored by one of the participating faculty; 2) a weekly seminar series in various aspects of cancer research, including breast cancer projects; and 3) a poster session and research forum at the end of the summer to highlight their research accomplishments. In the second year of the BCTP-SU, five outstanding students were recruited to the Eppley Institute. One student evaluated reagents for immunotherapy for breast cancer, two students studied genetic or epigenetic factors related to breast cancer, one student monitored cellular location of an important signed transduction modulator for breast cancer, and one student studied which cells in the pancreas serve as precursors of pancreatic cancer. All five students presented posters on their research. All five students are continuing on in research and/or medical fields, consistent with the goals of the BCTP-SU. Four of five BCTP-SU students were women, and one was a member of an under-represented minority.		
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DAMD17-01-1-0341
Breast Cancer Training Program for Summer Undergraduates
Kenneth H. Cowan, M.D., Ph.D., Principal Investigator
Annual Summary Report, Year 2: May 15, 2002-May 14, 2003

I. Introduction

The Breast Cancer Training Program for Summer Undergraduates (BCTP-SU) has been established within the Eppley Cancer Research Institute of the University of Nebraska Medical Center (UNMC). The purpose of the BCTP-SU is to expand the Eppley's summer undergraduate research program to add five additional students who will train specifically in breast cancer research. Trainees participate in didactic and academic activities including: 1) a 10 week, lab-intensive research project, mentored by one of the participating faculty; 2) a weekly seminar series in various aspects of cancer research, including breast cancer projects; and 3) a poster session and research forum at the end of the summer to highlight their research accomplishments. Recruitment of women and other under-represented minorities is a significant part of the undergraduate training.

II. Body

A. Student participants, their research projects, and their mentors

Five students for the BCTP-SU, plus six other students, were recruited from a pool of approximately 55 applicants. Thus the program is highly selective, admitting about 20% of the applicants. Students were selected based on their academic training, their statement of interest in cancer research, and letters of recommendation. Lab assignments were based in part on student preferences, from a list of available faculty mentors. All BCTP-SU students were assigned to training faculty identified in the original BCTP-SU application. Students arrived at UNMC on or about May 28 and stayed through August 2, for a total of 10 weeks. Table 1 shows the BCTP-SU student names, their undergraduate affiliations, their faculty mentors, and the title of their research projects.

Table 1. BCTP-SU students for reporting year

Student	Affiliation	Mentor	Title of research project
Jennifer Melander	Univ. Nebraska, Lincoln	Solheim	MHC Class I – Targeted Immunotherapies for Breast Cancer
Alicia Willette	Coll. St. Scholastica (MN)	Christman	Examination of Tumor Suppressor CpG Island Methylation in Breast Cancer Cell Lines
Alaina Oas	St. Mary's Univ. (MN)	Shull	Characterization of Emca-1 in BN x ACI Backcross Populations
Mary Ann Bleem	St. Louis Univ.	Lewis	Regulation of the Nucleo-cytoplasmic Distribution of KSR Protein, a Modulator of Ras Signalling
Rafael J Lopez	University of Puerto Rico - Cayey	Ouellette	Multipotent Nestin-Positive Stem Cells as a Precursor of Pancreatic Adenocarcinomas

B. Weekly seminars and other educational offerings

A mandatory weekly seminar was held to help educate the students about key areas in cancer research. Specific research results from the speakers' laboratories were used to illustrate concepts and to demonstrate how research is used to extend our knowledge about cancer. Two meetings were utilized for panel discussions with faculty and students regarding graduate research opportunities. The dates, speakers, and seminar titles are shown in Table 2.

Table 2. Mandatory seminars

Date	Speaker	Title
5/30/02	Robert Lahue, Ph. D. Elli Rogan, Ph. D.	Orientation and Safety
6/6/02	CRTP Students	CRTP Panel Discussion
6/13/02	Joyce Solheim, Ph. D.	The Cellular Immune Defense Against Tumors and Infections
6/20/02	Barry Gold, Ph. D.	DNA Damage and Repair
6/27/02	Margaret Wheelock, Ph.D.	Cellular junctions
7/4/02	No Seminar--Independence Day	
7/11/01	Michel Ouellette, Ph.D.	Graduate Research Opportunities
7/18/02	James Shull, Ph.D.	Cancer Genetics
7/25/02	Timothy McKeithan, Ph. D.	Transcriptional regulation of <i>BCL3</i> , a key mediator of the signal for immunological danger that determines immunity or tolerance
8/1/01	Summer Students	Poster Day Wrap-up meeting and invitation to apply to Eppley Graduate Program

In addition, students were encouraged to attend other campus seminars related to cancer research. These seminars were presented by visiting speakers, by Eppley Institute and UNMC faculty, and by Eppley graduate students. The list is shown in Table 3.

Table 3. Suggested seminars

Date	Speaker	Title
6/12/02	William G. Nelson, M.D., Ph.D	Molecular Pathogenesis of Prostate Cancer
6/25/02	Henry Lynch, M. D.	Familial Hematologic Cancer
07/1/02	Esta Sterneck, PhD	The C/EBPdelta transcription factor is a candidate tumor suppressor maintaining chromosome stability: Implications for lung and breast cancer
7/2/02	Steve Schreiner	Dissertation Defense
7/7/02	Andrei Khokhlatchev, PhD	Identification of a new Ras-regulated pathway that controls apoptosis
7/9/02	Xu Luo, PhD	Biochemical study of signaling pathways in apoptosis
7/14/02	Keshav Singh, PhD	Mitochondria, oxidative stress and genetic instability
7/24/02	Zheng Cui, MD, PhD	Lipids, Cancer and Immunosurveillance

C. Poster presentations

Each student presented a poster on her research at the UNMC summer student research forum, held Aug. 1. The student author, mentor, and title of each poster are shown in Table 1. Copies of the poster title, authors, and abstract are presented in the Appendix of this report.

D. Recruitment of women and other under-represented minorities

Four of five BCTP-SU trainees in the reporting year were female. One student (Lopez) is a member of an under-represented minority.

E. Tracking of trainees

The current status of each BCTP-SU trainee is shown in Table 4. Four of the students (Melander, Willette, Oas, and Bleem) have graduated from college. The other student (Lopez) will be a senior in the fall semester, 2003.

Table 4. Current status of trainees

Student	Educational Status	Awards, Honors, Current Situation
Jennifer Melander	Graduated	Received 2 nd place in the e-week poster presentations. Will attend graduate school at U. Nebraska-Lincoln in Biomedical Engineering
Alicia Willette	Graduated	Graduated with high honors. Currently working as a summer student at University of Minnesota and will attend dentistry school there.
Alaina Oas	Graduated	Honors program, Tri-Beta member, double major in chemistry and biology. Will attend medical school either in Belize, St. Luke's or St. John's
Mary Ann Bleem	Graduated	Won the Goldwater award and will be attending St. Louis University Medical Schoool
Rafael J Lopez	Rising senior	Deans List, won 2 nd place in spring research competition at Cayey University, Summer undergraduate research at UCLA

III. Key Research Accomplishments

- Melander et al studied the use of MHC1-related proteins, tapasin and invariant chain, as potential stimulators of breast cancer immunotherapy. They found that antigen presentation in breast cancer cells was effectively modulated by tapasin and invariant chain. These experiments provided the important proof-of-principle that tapasin and invariant chains are reasonable candidates to stimulate immunotherapy. Future experiments will focus on regulated and enhanced recognition of breast cancer cell peptides by cytolytic T lymphocytes.
- Willette et al examined DNA methylation in breast cancer cells to help identify novel methylated tumor suppressor genes. Methylation-specific quantitative PCR in MCF7 cells showed that methylation at the GSTP1 gene was inversely related to estrogen receptor status, but similar tests in MCH10A and MCH10AT cells did not support GSTP1 methylation status as a reliable indicator of ER status. In future experiments, additional genes, including BRCA1, will be targeted for interrogation of methylation status.
- Oas et al examined a gene called Emca-1, which has been correlated with sensitivity to estrogen-mediated breast cancer in rats. Genetic analysis of crosses between rat strains that were either sensitive or resistant to estrogen-mediated breast cancer supports a model where the Emca-1 allele from the sensitive rat strain is dominant for susceptibility.
- Bleem et al monitored the subcellular localization of KSR protein, which positively modulates Ras signalling, and which may be an important for promotion of breast cancer. The specific focus of this project was to better understand the nucleo-cytoplasmic distribution of KSR as a way to dissect its function. Mutants of KSR were prepared that lacked signals for nuclear export. Of two putative nuclear export signals, one (residues 42-57) were found to be essential for export. These findings are consistent with the idea that the subcellular localization of KSR is an important determinant of its function in Ras-mediated cell signalling.
- Lopez et al examined which cells in the pancreas serve as precursors to pancreatic cancers. Primary cultures from human pancreas were immortalized by introduction of the catalytic subunit of human telomerase (hTERT). The immortalized cell lines had unique characteristics of stem cells. To test the ability of these cells to differentiate to ductal epithelial cells, dexamethasone and sodium butyrate were shown to synergize in this differentiation. These cell lines should be extremely valuable in the future for additional studies on the cellular origin of pancreatic cancers.

IV. Reportable Outcomes

1. Each student presented his/her research at the UNMC Poster Day, August 1, 2002.
2. One student (Melander) has continued on in biomedical research. Three (Willette, Oas, and Bleem) will be attending dental or medical school. The fifth student (Lopez) is a rising senior.
3. Honors and awards for these students are detailed in Table 4.

V. Conclusions

In the second year of the BCTP-SU, five outstanding students were recruited to the Eppley Institute, where they performed research on breast cancer and other types of cancer. The students were highly motivated and successful in their projects. All five students have indicated their intention to continue on in research and/or medical fields, consistent with the goals of the BCTP-SU.

Recommendations for current and future years of the BCTP-SU are:

1. Continue to identify, recruit, and train outstanding undergraduate students.
2. Continue to focus on breast cancer research training for these students.
3. Continue to recruit women and other under-represented minorities.
4. Continue to maintain tracking of previous students.

MHC Class I – Targeted Immunotherapies for Breast Cancer

Jennifer Melander, Heth Turnquist, Adrian Reber, and Joyce Solheim

Breast cancer is a devastating disease, for which new, effective therapies are urgently needed. Accordingly, we are seeking to develop novel approaches to enhance cellular immune responses against breast cancer. Effective immune responses to cancer require that peptides from mutated or over-expressed proteins be presented on cell surface major histocompatibility complex (MHC) molecules to T lymphocytes, which recognize and lyse the abnormal cells. These tumor-specific or tumor-associated peptides are transported into the endoplasmic reticulum (ER) from the cytoplasm, and bind to MHC class I molecules in the endoplasmic reticulum. This process is complex and influenced by multiple ER proteins. One such protein is tapasin, a ubiquitously expressed MHC class I chaperone, which is proposed to be crucial for stable class I assembly with peptide. A second ER protein, invariant chain, which is normally only expressed in the antigen presenting cells of hematopoietic lineage, binds after MHC class I peptide-binding groove has folded and tapasin has been released. The influence of invariant chain on MHC class I antigen presentation is yet to be defined; however, evidence from our laboratory and others' indicates that invariant chain, as well as tapasin, may affect the repertoire of peptides that are presented by MHC class I molecules. Therefore, we have performed preliminary studies to test the feasibility of the administration of tapasin (wild type or mutant) and invariant chain as means to stimulate the presentation of breast tumor antigens to the immune system. First, our results indicate that transient transfection of tapasin molecules with specific site-directed mutations that have been designed to alter the tapasin class I interaction is a feasible method to study the effect of tapasin mutations on MHC class I interaction and tumor antigen presentation. Second, we found that introduction of invariant chain into breast tumor cells significantly increased the quantity of MHC class I molecules at the cell surface. Future studies will proceed to test the ability of tapasin, in both wild type and mutant form, to regulate the presentation of breast tumor peptides. In addition, future *in vitro* and *in vivo* experiments will pursue the effect of administered invariant chain on the presentation of specific breast tumor peptides and on the recognition of breast tumor cells by cytolytic T lymphocytes.

Examination of Tumor Suppressor CpG Island Methylation in Breast Cancer Cell Lines

Alicia Willette, Dana Van Bemmel, Michael Boland, Eva Uzvolgyi, Lin Tang and Judith K. Christman

Gene silencing in cancer is known to be associated with hypermethylation of CpG islands, which are usually located in the promoter or first exon region of a gene. CpG island methylation can result in tumor development due to silencing of associated tumor suppressor genes (1).

Currently little is known about the early changes in breast tissue that lead to tumor development. Successful completion of analysis of methylation changes in known genes (their activation and silencing), could lead to markers for early prognosis of breast cancer in women with a high risk for breast cancer development. In order to study CpG island methylation, we explored the methylation status of specific genes with breast cancer cell lines MCF7, MCF10A, MCF10AT. Methylation specific quantitative PCR was used to study the methylation status of GSTP1. GSTP1 methylation status has been claimed to be inversely related to estrogen receptor status in breast cancer. Our results for the MCF 7 cell line support this, however the MCF10A and MCF10AT do not. An attempt was made to examine methylation of BRCA1, TMS1, and GSTP1 by microarray. This technique could not be optimized due to time constraints. Profiling human breast cancer methylation status with these techniques has great potential for identification of novel methylated tumor suppressor genes and development of new prognostic tools.

Characterization of Emca-1 in BN x ACI Backcross Populations

Alaina J. Oas, Beverly S. Schaffer, and James Shull

Estrogen plays a major role in the development and progression of breast cancer. When treated continuously with 17b-estradiol (E2), female ACI rats are highly susceptible to the development of mammary cancer. In contrast, Brown Norway (BN) rats are highly resistant to the development of E2 induced mammary cancer. In reciprocal crosses between the ACI and BN strains, a locus, called Emca-1 was identified on chromosome five that plays a role in E2-induced mammary cancer. To understand better the genetics of E2-induced mammary cancer, the Emca-1 locus was characterized in backcross (BC) populations generated by crossing an (ACI x BN)F1 female back to either an ACI male (BCa) or a BN male (BCb). To determine genotypes at the Emca-1 locus, DNA was purified from spleen and analyzed by polymerase chain reaction (PCR) using polymorphic markers. The genotypic and phenotypic data were then analyzed using MAPMAKER/QTL. Phenotypes of latency, tumor positive at 175 days and 196 days, number of tumors, and tumor volume were analyzed. Analysis of the BCa population supports a model in which the ACI allele of Emca-1 acts in a dominant manner to determine susceptibility to E2-induced mammary cancer. Data from the BCb population are inconsistent with this model, perhaps because very few rats in the BCb population developed mammary cancer when treated with E2.

Regulation of the Nucleo-cytoplasmic Distribution of KSR Protein, a Modulator of Ras Signalling

Mary Ann Bleem, Gina Razidlo and Robert Lewis

Kinase Suppressor of Ras (KSR) is a putative molecular scaffold in the Ras/Raf/MEK/ERK kinase cascade and a positive regulator of the pathway. Activating mutations of Ras have been found in a number of human cancers, including breast, colon, and lung cancer. KSR is localized to the cytoplasm of quiescent cells, but cycles continuously through the nucleus. In addition, KSR accumulates in the nucleus upon stimulation. Disrupting the regulation of the scaffold's nucleo-cytoplasmic distribution alters the cell's response to mitogenic stimulation. CRM1-dependent nuclear export is regulated by the presence of a nuclear export signal (NES). Residues 42-57 and residues 822-837 of KSR both exhibit homology with other leucine-rich sequences used for CRM1 mediated export. These regions were deleted independently and in combination and the nucleo-cytoplasmic distribution of KSR was evaluated. KSRD42-57 is redistributed to the nucleus under steady state conditions, whereas KSRD822-837 is seen in the cytoplasm. Combining the two mutated sequences also results in cytoplasmic distribution. As wild type KSR is localized to the cytoplasm, this suggests that residues 42-57 are involved in nuclear export. To further verify this, Ref-52 cells transiently transfected with GFPKSRD42-57 were treated with wheat germ agglutinin, an inhibitor of nuclear import. Upon treatment, KSRD42-57 accumulates in the cytoplasm, suggesting that it is still able to be exported from the nucleus. Regulation of the nucleo-cytoplasmic distribution of a molecular scaffold is critical in determining the mechanism and biological outcome of a mitogenic signaling pathway. Identification of a nuclear export signal in a scaffold is a crucial step in understanding the subcellular distribution of the protein, as well as other components in the signaling pathway.

Multipotent Nestin-Positive Stem Cells as a Precursor of Pancreatic Adenocarcinomas

Rafael J. Lopez, Kwangmoon Lee, Hiroaki Yasuda, Michael A. Hollingsworth, Parviz M. Pour, & Michel M. Ouellette

Pancreatic adenocarcinomas are the fourth most common cause of cancer death in the Western World. At the time of diagnosis, 80% of these tumors have already spread beyond the gland to regional lymph nodes, the liver and other sites. Pancreatic adenocarcinomas originate from within the duct system of the exocrine pancreas and are classified as ductal on the basis of their histological appearance. However, the exact nature of the cell type from which these tumors arise is still a matter of controversy. The proposed candidates include the ductal cells, the acinar cells and the putative stem cells of the pancreas. The goal of the present study is to verify the possibility that adult stem cells exist in the pancreas that can serve as precursors to pancreatic cancers. We have used the catalytic subunit of human telomerase (hTERT) to immortalize primary cultures derived from ducts of a human pancreas. The procedure has led to the establishment of the hTERT-HPNE cells, a unique line with stem cell characteristics. The immortalized cells express many markers of neuronal stem cells, which include nestin, MDR1 and members of the Notch pathway. Nestin-positive cells have been detected by others in the ducts and islets of the adult pancreas and their data suggest that the cells can serve as multipotent stem cells to all of the major pancreatic lineages. In the present proposal, we plan on testing the hTERT-HPNE cells under various culture conditions to verify their capacity to give rise to pancreatic ductal epithelial cells. Our results suggest that dexamethasone (1 mM) and sodium butyrate (10 mM) synergize to promote the conversion of the cells to such a phenotype, as indicated by the expression of CK7 and CK19.